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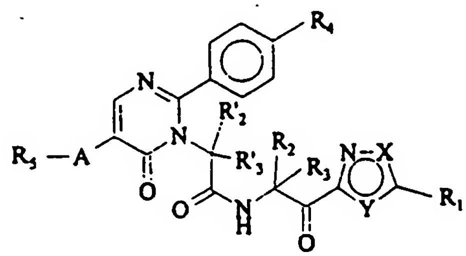
(54) Title: ALPHA-KETO OXADIAZOLES AS SERINE PROTEASE INHIBITORS

(57) Abstract

The present invention relates to certain substituted oxadiazole, thiadiazole, and triazole peptoids containing valine and proline residues and nonpeptoids containing pyrimidinone residues useful as inhibitors of serine proteases, for example human neutrophil elastase (HNE). Compounds of the present invention are useful for the treatment or amelioration of symptoms of adult respiratory distress syndrome, septic shock, and multiple organ failure. Processes mediated by HNE are also implicated in conditions such as arthritis, periodontal disease, glomerulonephritis, and cystic fibrosis.

In another embodiment, the present invention provides compounds of formula II:



II

wherein X and Y are independently O or N;

R_1, R_2, and R_3 are as above;

R'_2 and R'_3 are independently H or alkyl; or together form a ring consisting of 3-5 carbon atoms in which one or more carbon atoms of the ring can optionally be replaced by heteroatoms selected from O, S or N,

wherein N is optionally substituted with H or alkyl;

A is a direct bond, -NH- or -OC(O)-NH-;

R_4 is H or halo; and

R_5 is H, alkyl or arylalkyl; or

a pharmaceutically acceptable salt thereof.

Preferably, compounds of this embodiment of the present invention comprise a 1,3,4 oxadiazole ring (i.e., X is N; Y is O).

In one preferred embodiment of the invention, R_1 is alkyl, such as *tert*-butyl. In another embodiment, R_1 is α,α-dialkylalkyl fused aryl-cycloalkyl wherein the cycloalkyl group is substituted with two O atoms, such as an α,α-dimethyl-(3,4-methylenedioxy)benzyl group. In yet another embodiment, R_1 is α,α-dialkylalkylaryl, such as an α,α-dimethylbenzyl group. In still another preferred embodiment, R_2 and R_3 are independently alkyl, such as isopropyl, or H. In a more preferred embodiment, R_2 is isopropyl, R_3 is H, and R_2' and R_3' are both H. Where R_4 is halo, R_4 may be Cl, F, I or Br, although preferably it is F.

As used herein, the term "optionally substituted" means, when substituted, mono to fully substituted.

ALPHA-KETO OXADIAZOLES AS SERINE PROTEASE INHIBITORS

This application claims the benefit of the filing date of U.S. Serial No. 09/090,046and U.S. Serial No. 09/090,274, filed June 3, 1998; both of which are incorporated herein by reference.

Background of the Invention

The serine proteases are a class of enzymes, which includes elastase, chymotrypsin, cathepsin G, trypsin and thrombin. These proteases have in common a catalytic triad consisting of Serine-195, Histidine-57 and Aspartic acid-102(chymotrypsin numbering system). Human neutrophil elastase (HNE) is a proteolytic enzyme secreted by polymorphonuclear leukocytes (PMNs) in response to a variety of inflammatory stimuli. This release of HNE and its extracellular proteolytic activity are highly regulated and are normal, beneficial functions of PMNs. The degradative capacity of HNE, under normal circumstances, is modulated by relatively high plasma concentrations of α_1-proteinase inhibitor (α-PI). However, stimulated PMNs produce a burst of active oxygen metabolites, some of which (hypochlorous acid for example) are capable of oxidizing a critical methionine residue in α-PI. Oxidized α-PI has been shown to have limited potency as an HNE inhibitor and it has been proposed that alteration of this protease/antiprotease balance permits HNE to perform its degradative functions in localized and controlled environments.

Despite this balance of protease/antiprotease activity, there are several human disease states in which a breakdown of this control mechanism is implicated in the pathogenesis of the condition. Improper modulation of HNE activity has been suggested as a contributing factor in adult respiratory distress syndrome, septic shock and multiple organ failure. A series of studies also have indicated the involvement of PMNs and neutrophil elastase in myocardial ischemia-reperfusion injury. Humans with below-normal levels of α_1-PI have an increased probability of developing emphysema. HNE-mediated processes are implicated in other conditions such as arthritis, periodontal disease, glomerulonephritis, dermatitis, psoriasis, cystic fibrosis, chronic bronchitis, atherosclerosis, Alzheimer's disease, organ transplantation, corneal ulcers, and invasion behavior of malignant tumors.

There is a need for effective inhibitors of HNE as therapeutic and as prophylactic agents for the treatment and/or prevention of elastase-mediated problems.

degradative effects associated with the presence of HNE. Their usage is of particular importance as they relate to various human treatment *in vivo* but may also be used as a diagnostic tool *in vitro*.

The present invention provides, but is not limited to, specific embodiments set forth in the Examples as well as those set forth below.

The nomenclature for the embodiments is as follows (although embodiments disclosed indicate the stereochemistry of the 2-methylpropyl group as having the *(S)*-configuration, it will be understood that both the enantiomerically pure *(R)* and racemic *(R,S)* configurations are within the scope of the invention):

Example 1 Methyloxycarbonyl-*L*-valyl-*N*-[1-(2-[5-(*tert*-butyl)-oxadiazolyl]carbonyl)-2-*(S)*-methylpropyl]-*L*-prolinamide.

Example 2 Methyloxycarbonyl-*L*-valyl-*N*-[1-(2-[5-(α,α-dimethylbenzyl)-oxadiazolyl]carbonyl)-2-*(S)*-methylpropyl]-*L*-prolinamide.

Example 3 Methyloxycarbonyl-*L*-valyl-*N*-[1 -(2-[5-(α,α-dimethyl-3,4-methylenedioxybenzyl)- 1,3,4-oxadiazolyl]carbonyl)-2-*(S)*-methylpropyl]-*L*-prolinamide.

Example 4 2-[6-Oxo-2-(4-fluorophenyl)-1,6-dihydro-1-pyrimidinyl]-N-[l-(2-[5-*tert*-butyl 1,3,4-oxadiazolyl]carbonyl)-2-(S)-methylpropyl]acetamide.

Example 5 2-[5-Benzyloxycarbonylamino-6-oxo-2-(4-fluorophenyl) 1,6-dihydro-1- pyrimidinyl]-N-[1-(2-[5-(α,α-dimethyl-3,4-methylenedioxybenzyl)-1,3,4-oxadiazolyl]carbonyl)-2-(*S*)-methylpropyl]acetamide.

Example 6 2-[5-Amino-6-oxo-2-(4-fluorophenyl)-1,6-dihydro-l-pyrimidinyl]-N-[1-(2-[5-(α,α-dimethyl-3,4-methylenedioxybenzyl)-1,3,4-oxadiazolyl]carbonyl)-2-(*R,S*)-methylpropyl]acetamide.

Example 7 2-[5-Benzyloxycarbonylamino-6-oxo-2-phenyl-1,6-dihydro-1-pyrimidinyl]-N-[l-(2-[5-(α,α-dimethyl-3,4-methylenedioxybenzyl)-1,3,4-oxadiazolyl]carbonyl)-2-(S)-methylpropyl]acetamide.

Example 8 2-[5-Amino-6-oxo-2-phenyl-1,6-dihydro-l-pyrimidinyl]-N-[l-(2-[5-(α,α-dimethyl-3,4methylenedioxybenzyl)-1,3,4-oxadiazolyl]carbonyl)-2-(*R,S*)-methylpropyl]acetamide.

The characteristics for the P_1 residue defining serine proteinase specificity is well established. The proteinases may be segregated into three subclasses: elastases, chymases and tryptases based on these differences in the P_1 residues. The elastases prefer small aliphatic moieties such as valine whereas the chymases and tryptases prefer large aromatic hydrophobic and positively charged residues respectively.

One additional proteinase that does not fall into one of these categories is propyl endopeptidase. The P_1 residue defining the specificity is a proline. This enzyme has been implicated in the progression of memory loss in Alzheimer's patients. Inhibitors consisting of α-keto heterocycles have recently been shown to inhibit propyl endopeptidase (Tsutsumi et al., *J. Med. Chem.*, 37, 3492-3502 (1994)). By way of extension, α-keto heterocycles as defined herein allow for an increased binding in P' region of the enzyme.

Table 1. P_1 Characteristics for Proteinase Specificity

Proteinase Class	Representative Enzyme	P_1 Characteristic
Elastases	Human Neutrophil Elastase	small aliphatic residues
Chymases	alpha- Chymotrypsin, Cathepsin G	aromatic or large hydrophobic residues
Tryptases	Thrombin, Trypsin, Urokinase, Plasma Kallikrein, Plasminogen Activator, Plasmin	positively charged residues
Other	Prolyl Endopeptidase	proline

Since the P_1 residue predominately defines the specificity of the substrate, the present invention relates to P_1-P_n' modifications, specifically, certain alpha-substituted keto-heterocycles composed of 1,2,4 oxadiazoles and 1,3,4-oxadiazoles. By altering the alpha-substituent to the ketone and, to some extent, the substituent on the heterocycle, the specificity of these compounds can be directed toward the desired proteinase (e.g., small aliphatic groups for elastase).

The efficacy of the compounds for the treatment of various diseases can be determined by scientific methods, which are known in the art. The following are noted as examples for HNE mediated conditions:

- for acute respiratory distress syndrome, the method according to human neutrophil elastase (HNE) model *(AARD*, 141:227-677 (1990)); the endotoxin induced acute lung injury model in minipigs *(AARD*, 142:782-788 (1990)); or the method according to human

Organic Chemistry (M. Hudlicky, ACS Monograph 186 (1990)) yields the desired ketone (9) or (9').

Where a compound is substituted at the 5 position of the pyrimidinone group with a benzyloxycarbonylamino group, a deprotection step can be conducted as described in Figure 3. This step requires removal of the protecting group from the amine and may be carried out by a number of methods. For example, one may utilize aluminum chloride, anisole and nitromethane in a suitable solvent such as dichloromethane to give the 5-amino compound (10'). Other methods of deprotection available in the art may also be used.

Although the compounds described herein may be administered as pure chemicals, it is preferable to present the active ingredient as a pharmaceutical composition. The invention thus further provides the use of a pharmaceutical composition comprising one or more compounds together with one or more pharmaceutically acceptable carriers thereof and, optionally, other therapeutic and/or prophylactic ingredients. The carrier(s) must be 'acceptable' in the sense of being compatible with the other ingredients of the composition and not deleterious to the recipient thereof.

Pharmaceutical compositions include those suitable for oral or parenteral (including intramuscular, subcutaneous and intravenous) administration. The compositions may, where appropriate, be conveniently presented in discrete unit dosage forms and may be prepared by any of the methods well known in the art of pharmacy. Such methods include the step of bringing into association the active compound with liquid carriers, solid matrices, semi-solid carriers, finely divided solid carriers or combination thereof, and then, if necessary, shaping the product into the desired delivery system.

Pharmaceutical compositions suitable for oral administration may be presented as discrete unit dosage forms such as hard or soft gelatin capsules, cachets or tablets each containing a predetermined amount of the active ingredient; as a powder or as granules; as a solution, a suspension or as an emulsion. The active ingredient may also be presented as a bolus, electuary or paste. Tablets and capsules for oral administration may contain conventional excipients such as binding agents, fillers, lubricants, disintegrants, or wetting agents. The tablets may be coated according to methods well known in the art, e.g., with enteric coatings.

Oral liquid preparations may be in the form of, for example, aqueous or oily suspension, solutions, emulsions, syrups or elixirs, or may be presented as a dry product for constitution with water or other suitable vehicle before use. Such liquid preparations may

the age and condition of the patient and will be ultimately at the discretion of the attendant physician or clinician.

In general, however, a suitable dose will be in the range of from about 0.5 to about 100 mg/kg/day, e.g., from about 1 to about 75 mg/kg of body weight per day, such as 3 to about 50 mg per kilogram body weight of the recipient per day, preferably in the range of 6 to 90 mg/kg/day, most preferably in the range of 15 to 60 mg/kg/day.

The compound is conveniently administered in unit dosage form, for example, containing 0.5 to 1000 mg, conveniently 5 to 750 mg, and most conveniently, 10 to 500 mg of active ingredient per unit dosage form.

Ideally, the active ingredient should be administered to achieve peak plasma concentrations of the active compound of from about 0.5 to about 75 μM, more preferably, about 1 to 50 μM, and most preferably, about 2 to about 30 μM. This may be achieved, for example, by the intravenous injection of a 0.05 to 5% solution of the active ingredient, optionally in saline, or orally administered as a bolus containing about 0.5-500 mg of the active ingredient. Desirable blood levels may be maintained by continuous infusion to provide about 0.01-5.0 mg/kg/hr or by intermittent infusions containing about 0.4-15 mg/kg of the active ingredient(s).

The desired dose may be conveniently presented in a single dose or as divided doses administered at appropriate intervals, for example, as two, three, four or more sub-doses per day. The sub-dose itself may be further divided, e.g., into a number of discrete loosely spaced administrations, such as multiple inhalations from an insufflator or by application of a plurality of drops into the eye.

While the invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications and this application is intended to cover any variations, uses, or adaptations of the invention following, in general, the principles of the invention and including such departures from the present disclosure as come within known or customary practice within the art to which the invention pertains and as may be applied to the essential features hereinbefore set forth, and as follows in the scope of the appended claims.

The following examples are given to illustrate the invention and are not intended to be inclusive in any manner.

under reduced pressure. The residue was azeotroped with toluene several times, dissolved in a saturated aqueous solution of sodium chloride, and extracted with chloroform (4x). The extract was dried over anhydrous sodium sulfate, and concentrated under reduced pressure to give *tert*-butylcarbohydrazonic acid (176 g) having the following physical data.

TLC: R_f = 0.59, chloroform: methanol (10:1).

^{1}H NMR (DMSO-d_6): δ8.78 (1H, brs), 4.15 (2H, brs), 1.08 (9H, s).

B. 2-*tert*-Butyl-1,3,4-oxadiazole

The mixture consisting of *tert*-butylcarbohydrazonic acid (176 g), trimethyl orthoformate (250 ml) and p-toluenesulfonic acid monohydrate (4.3 g) was heated and methanol removed by distillation at a temperature ranging from 90°C to 110°C. Trimethyl orthoformate was removed (50°C/43 mm Hg) and the residue was distilled at 120°C/23 mm Hg to give 2-*tert*-Butyl-1,3,4-oxadiazole (131 g) having the following physical data.

TLC: R_f= 0.68, chloroform: methanol (10:1).

^{1}H NMR (DMSO-d_6): δ9.12(1 H,s), 1.3 6 (9H, s).

C. 1-[2-(5-*tert*-Butyl)-1,3,4-oxadiazolyl]-2-*(S)*-(*tert*-butoxycarbonylamino)-3-methylbutan-1-ol

To a solution of 2-*tert*-Butyl-1,3,4-oxadiazole (62.1 g) in tetrahydrofuran (1650 ml) was added *n*-butyllithium in hexane (1.6 M, 307.8 ml) dropwise at -78°C under an atmosphere of argon. The mixture was stirred for 40 min at -78°C, magnesium bromide diethyl etherate (127.2 g) was added, and the resulting mixture was allowed to warm to -45°C. After 1.5 hours, a solution of 2-(S)-[N-(*tert*-butoxycarbonyl)amino]-3-methylbutanal (90 g) in tetrahydrofuran (60 ml) was added dropwise at -45°C and allowed to warm to -15°C. The reaction mixture was quenched by addition of a saturated aqueous solution of ammonium chloride, and extracted with ethyl acetate. The extract was washed with water (x3) and a saturated aqueous solution of sodium chloride, dried over anhydrous sodium sulfate and concentrated. The residue was purified by column chromatography on silica gel (Merck 7734) (ethyl acetate:hexane = 1:20 to 1:1) to give 1-[2-(5-*tert*-butyl)-1,3,4-oxadiazolyl]-2-*(S)*-(*tert*-butoxycarbonylamino)-3-methylbutan-1-ol (78.6 g) having the following physical data.

TLC: R_f = 0.42, hexane:ethyl acetate (1:1).

^{1}H NMR ($CDCl_3$): δ5.16-4.90 (2H, m), 4.67 (1H, m), 4.23 (1H, m), 3.90 (1H, m), 3.66 (1H, m), 1.98 (1H, m), 1.42, 1.41 and 1.36 (total 18H, each s), 1.13-0.90 (6H, m).

D. 1-[2-(5-*tert*-Butyl)-1,3,4-oxadiazolyl]-2-*(S)*-amino-3-methylbutan-1-ol Hydrochloride

The product had the following physical data.

TLC: $R_f = 0.64$, ethyl acetate.

1H NMR (200 MHz, $CDCl_3$): 7.84 and 7.49 (each brd., J=7.6 Hz, totally 1H, NH), 7.40-7.20 (m, 5H aromatic Hs), 5.46-5.29 (m, 2H, NH and α CH of P_1 Val), 4.77-4.60 (m, 1H, α CH of Pro), 4.40-4.25 (m, 1H α CH of P_3 Val), 3.84-3.55 (m, 2H, NCH_2 of Pro), 3.68 (s, 3H, CH_3O), 2.55-1.76 (m, 6H, CHs of *iso*-Pr andCH_2CH_2 of Pro), 1.88 (s, 6H, het$C(CH_3)_2Ph$), 1.12-0.82 (m, 12H, CH_3s of *iso*-Pr).

Example 3 Methyloxycarbonyl-*L*-valyl-*N*-[1 -(2-[5-(α,α-dimethyl-3,4-methylene-dioxybenzyl)- 1,3,4-oxadiazolyl]carbonyl)-2-*(S)*-methylpropyl]-L-prolinamide

The compound was prepared by oxidizing methyloxycarbonyl-*L*-valyl-*N*-[1-(2-[5-(α,α-dimethyl-3,4-methylenedioxybenzyl)-oxadiazolyl]hydroxymethyl)-2-*(S)*-methylpropyl]-*L*-prolinamide using a procedure know to one skilled in the art, such as, the Swern Oxidation.

The intermediate, methyloxycarbonyl-*L*-valyl-*N*-[1-(2-[5-(α,α-dimethyl-3,4-methylenedioxybenzyl)-oxadiazolyl]hydroxymethyl)-2-*(S)*-methylpropyl]-*L*-prolinamide, was prepared using methyloxycarbonyl-*L*-Val-Pro-OH and l-[2-(α,α-dimethyl-3,4-methylenedioxybenzyl)-1,3,4-oxadiazolyl]-2-*(S)*-amino-3-methylbutan-l-ol hydrochloride and a coupling method know to one skilled in the art. The intermediate 1-[2-(α,α-dimethyl-3,4-methylenedioxybenzyl)-1,3,4-oxadiazolyl]-2-*(S)*-amino-3-methylbutan-1-ol hydrochloride was prepared using a similar procedure as described in Example 1 except methyl 3,4-methylenedioxyphenylisobutyrate was used instead of methyl trimethylacetate.

The product had the following physical data.

TLC: Rf = 0.63, ethyl acetate.

[1]H NMR (200 MHz, $CDCl_3$): 7.49 (d, J=6.4 Hz, 1H, NH), 6.85-6.73 (m, 3H, aromatic Hs), 5.95 (s, 2H, OCH_2O), 5.46-5.28 (m, 1H α CH of Pro), 4.30 (m, 1H, α CH of P_3-Val), 3.84-3.54 (m, 2H, NCH_2 of Pro), 3.68 (s, 3H, CH_3O), 2.55-1.78 (m, 6H, CHs of *iso*-Pr, and CH_2CH_2 of Pro), 1.83 (s, 6H, Het$C(CH_3)2Ph$), 1.11-0.85 (m, 12H, CH_3s of *iso*-Pr).

Example 4 2-[6-Oxo-2-(4-fluorophenyl)-1,6-dihydro-l-pyrimidinyl]-N-[1-(2-[5-*tert*-butyl-1,3,4-oxadiazolyl]carbonyl)-2-(*S*)-methylpropyllacetamide

To a solution of oxalyl chloride (5.80 ml) in dichloromethane (160 ml) was slowly added dropwise a solution of dimethylsulfoxide (9.44 ml) in dichloromethane (16 ml) at –78°C under an atmosphere of argon. The mixture was stirred for 30 min at 78°C. To the mixture was added dropwise a solution of 2-[6-Oxo-2-(4-fluorophenyl)-1,6-dihydro-l-pyrimidinyl]-N-

After 1.5 hours, a solution of 2-(S)-[N-(*tert*-butoxycarbonyl)amino]-3-methylbutanal (9O g) in tetrahydrofuran (60 ml) was added dropwise at –45°C and allowed to warm to –15°C. The reaction mixture was quenched by addition of a saturated aqueous solution of ammonium chloride, and extracted with ethyl acetate. The extract was washed with water (x3) and a saturated aqueous solution of sodium chloride, dried over anhydrous sodium sulfate and concentrated. The residue was purified by column chromatography on silica gel (Merck 7734) (ethyl acetate:hexane = 1:2O → 1:1)

to give 1-[2-(5-*tert*-butyl)-1,3,4-oxadiazolyl]-2-(*S*)-(*tert*-butoxycarbonylamino)-3-methylbutan-1-ol (78.6 g) having the following physical data.

TLC: R_f = 0.42, hexane:ethyl acetate (1:1).

^{1}H NMR ($CDCl_3$): δ 5.16-4.90 (2H, m), 4.67 (1H, m), 4.23 (1H, m), 3.90 (1H, m), 3.66 (1H, m), 1.98 (1H, m), 1.42, 1.41 and 1.36 (total 18H, each s), 1.13-0.90 (6H, m).

D. 1-[2-(5-*tert*-Butyl)-1,3,4-oxadiazolyl]-2-(*S*)-amino-3-methylbutan-1-o1 Hydrochloride

To a solution of 1-[2-(5-*tert*-butyl)-1,3,4-oxadiazolyl]-2-(*S*)-(*tert*-butoxycarbonylamino)-3-methylbutan 1-ol (76.3 g) in dioxane (200ml) was added 4N hydrochloric acid in dioxane solution (1000 ml) at 0°C. The reaction mixture was concentrated under reduced pressure. The residue was solidified with diethyl ether. The solid was azeotroped with benzene several times to give l-[2-(5-*tert*-butyl)-l,3,4-oxadiazolyl]-2-(S)-ainino-3-methylbutan-1-ol hydrochloride (66.1 g) having the following physical data.

TLC: R_f= 0.30, chloroform:methanol (10:1);

^{1}H NMR ($CDCl_3$): δ 8.50-8.10 (2H, br), 7.10-6.80 (1 H, br), 5.55-5.35 (1H, m), 3.95-3.60 (2H, m), 2.10 (1H, m), 1.41 (9H, s), 1.20-1.00 (6H, m).

E. 2-[6-Oxo-2-(4-fluorophenyl)-1,6-dihydro-l-pyrimidinyl]-N-[1-(2-[5-*tert*-butyl-1,3,4-oxadiazol]hydroxymethyl-)2-(*S*)-methylpropyl]acetamide

To a solution of 1-[2-(5-*tert*-butyl)-1,3,4-oxadiazolyl]-2-(*S*)-amino-3-methylbutan-1-ol hydrochloride (10.76 g), [6-oxo-2-(4flurophenyl)-1,6-dihydro-1-pyrimidinyl]acetic acid (8.63 g) and 1-hydroxybenzotriazole (5.85 g) in dimethylformamide (100 ml) was added 1-ethyl-3-[3-(dimethylamino) propyl]carbodiimide (7.33 g) at 0°C. To the resulting mixture was added 4-methylmorpholine (4.21 ml) at the same temperature. The reaction mixture was stirred for 17 hours at room temperature. The reaction was quenched by addition of water, extracted with ethyl acetate (x3). The extract was washed with aqueous 10% citric acid solution, a saturated aqueous solution of sodium hydrogencarbonate, water and a saturated aqueous solution of sodium chloride. The organic phase was dried over anhydrous sodium

Example 1 D. The heterocyclic intermediate 2-(α,α-dimethyl-3,4-methylenedioxybenzyl)-1,3,4-oxadiazole gave the following physical data.

TLC: R_f = 0.69, chloroform:methanol (10:1).

^{1}H NMR ($CDCl_3$): δ 8.30 (1H, s), 6.78(1H, brs), 6.74(2H, brs), 5.94 (2H, s), 1.81 (6H, s).

Example 6 2-[5Amino-6-oxo-2-(4-fluorophenyl)-1,6-dihydro-l-pyrimidinyl]-N-[1-(2-[5-(α,α-dimethyl-3,4-methylenedioxybenzyl)-1,3,4-oxadiazolyl]carbonyl)-2-(*R,S*)-methylpropyl]acetamide

To 2-[5-(benzyloxycarbonylamino)-6-oxo-2-(4-fluorophenyl)-1,6-dihydro-l-pyrimidinyl]-N-[1-(2-[5-(α,α-dimethyl-3,4-methylenedioxybenzyl)-1,3,4-oxadiazolyl]carbonyl)-2-(*S*)-methylpropyl]acetamide (1.42 g) was added 30% hydrobromic acid in acetic acid solution (50 ml). The reaction mixture was stirred for I hour at room temperature. The reaction mixture was quenched by addition of ice water, extracted with ethyl acetate (x2). The combined extracts were washed with water (x2) and a saturated aqueous solution of sodium chloride. The organic phase was dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel using a gradient elution of 50 to 100% ethyl acetate/hexane to give 2-[5-amino-6-oxo-2-(4-fluorophenyl)-1,6-dihydro-1- pyrimidinyl]-N-[1-(2-[5-(α,α-dimethyl-3,4-methylenedioxybenzyl)-1,3,4-oxadiazolyl]carbonyl)-2-(*R,S*)-methylpropyl]acetamide (457 mg) having the following physical data.

TLC: R_f= 0.39, ethyl acetate.

^{1}H NMR ($CDCl_3$): δ 7.53 (2H, dd, J=8.8, 5.3Hz), 7.48 (1H, s), 7.06 (2H, t, J=8.8Hz), 6.90 (1H, brd, J=8.4Hz), 6.84-6.70 (3H, m), 5.95 (2H, s), 5.43 (1H, dd, J=8.4, 4.8 Hz), 4.63 and 4.54 (each 1H Abq, J=15.0Hz), 4.05 ((2H, brs), 2.51 (1H, m), 1.84 (6H, s), 1.06 and 0.87 (each 3H, each d, J=7.0Hz).

Example 7 2-[5-Benzyloxycarbonylamino-6-oxo-2-pheny-l-1,6-dihydro-l-pyrimidinyl]-N-[1-(2-[5-(α,α-dimethyl-3,4-methylenedioxybenzyl)-1,3,4-oxadiazolyl]carbonyl)-2-(*R,S*)-methylpropyl]acetamide

The compound was prepared using a similar oxidative procedure as described in Example 1 utilizing 2-[5-benzyloxycarbonylamino-6-oxo-2-phenyl-1,6-dihydro-l-pyrimidinyl]-N-[l-(2-[-5(α,α-dimethyl-3,4methylenedioxybenzyl)-1,3,4-oxadiazolyl]hydroxymethyl)-2-(*S*)-methylpropyljacetamide for the 2° alcohol. The title compound, 2-[5-benzyloxycarbonylamino-6-oxo-2-phenyl-1,6-dihydro-l-pyrimidinyl]-N-[1-(2-[5-(α,α-dimethyl-3,4-

Example 9 2-[6-oxo-2-phenyl-1,6-dihydro-l-pyrimidinyl]-N-[1-(2-[5-(α,α-dimethyl-3,4-methylenedioxybenzyl)-1,3,4-oxadiazolyllcarbonyl)-2-(*R,S*)-methylpropyl] acetamide

TLC: R_f = 0.46, ethyl acetate.

^{1}H NMR ($CDCl_3$): δ 8.01 (1H, d, J=6.6Hz), 7.65-7.35 (5H, m), 6.87 (1H, d, J=8.6,Hz), 6.85-6.70 (3H, m), 6.49 (1H, d, J=6.6Hz), 5.95 (2H, s), 5.42 (1H, dd, J=8.6 and 5.0Hz), 4.67 (1H, d, J=15.2Hz), 4.54 (1H, d, J=15.2Hz), 2.63-2.37 (1H, m), 1.84 (6H, s), 1.05 (3H, d, J=6.8Hz), 0.85 (3H, d, J=6.8Hz)

Example 10 2-[6-oxo-2-(4-fluorophenyl)-1,6-dihydro-l-pyrimidinyl]-N-[1-(2-[5-(α,α-dimethyl-3,4-methylenedioxybenzyl)-1,3,4-oxadiazolyl]carbonyl)-2-(*R,S*)-methylpropyl] acetamide

TLC: R_f = 0.43, ethyl acetate.

^{1}H NMR ($CDCl_3$): δ 7.99 (1H, d, J=6.6Hz), 7.63 (2h, dd, J=8.6, 5.2Hz), 7.14 (2H, t, J=8.6Hz), 6.93 (1H, brd, J=8.6Hz), 6.84-6.70 (3H, m), 6.49 (1H, d, J=6.6Hz), 5.95 (2H, s), 5.41 (1H, dd, J=8.6, 5.0Hz), 4.64 and 4.53 (each 1H, Abq, J= I 5.0Hz), 2.50 (1H, m), 1.84 (6H, s), 1.06 and 0.87 (each 3H, each d, J=7.0Hz).

Example 11 2-[6-oxo-2-(4-fluorophenyl-)1,6-dihydro-l-pyrimidinyl]-N-[1-(2-[5-(α,α-dimethylbenzyl)-1,3,4-oxadiazolyl]carbonyl)-2-(*R,S*)methylpropyl]acetamide

The compound was prepared using a similar oxidative procedure as described in Example 1 utilizing 2-[6-oxo-2-(4-fluorophenyl)-1,6-dihydro-l-pyrimidinyl]-N-[1-(2-[5-(α,α-dimethylbenzyl)-1,3,4-oxadiazolyl]hydroxymethyl)-2-(*S*)-methylpropyl]acetamide for the 2° alcohol. The title compound, 2-[6-oxo-2-(4-fluorophenyl)-1,6-dihydro-l-pyrimidinyl]-N-[1-(2-[5-(α,α-dimethylbenzyl)-1,3,4-oxadiazolyl]carbonyl)-2-(*S*)-methylpropyl]acetamide, gave the following physical data.

TLC: R_f = 0.42, ethyl acetate.

^{1}H NMR ($CDCl_3$): δ 7.99 (1H, d, J=6.5Hz), 7.62 (2H, m), 7.40-7.20 (5H, m), 7.14 (2H, t, J=8.8Hz), 6.89 (1H, brd, J=8.6Hz), 6.49 (1H, d, J=6.5Hz), 5.42 (1H, dd, J=8.6, 5.0Hz), 4.61 and 4.54 (each 1H, each d, J=15.0Hz), 2.50 (1H, m), 1.88 (6H, s), 1.06 and 0.86 (each 3H, each d, J=6.7Hz).

The intermediate 2-[6-oxo-2-(4-fluorophenyl)-1,6-dihydro-l-pyrimidinyl]-N-[1-(2-[5-(α,α-dimethylbenzyl)-1,3,4-oxadiazolyl]hydroxymethyl)-2-(*S*)-methylpropyl]acetamide was prepared in an analoguous manner as described in Example 1 E using [6-oxo-2-(4-fluorophenyl)1,6-dihydro-1-pyrimidinyllacetic acid and 1-[2-(α,α-dimethylbenzyl)-1,3,4-oxadiazolyl]-2-

1H, CH of *iso*-Pr), 1.48 (s, 9H, CH_3s of *t*-Bu), 1.07 and 0.88 (each d, J=6.8 Hz, each 3H, CH3s of *iso*-Pr).

Example 14 — In Vitro Inhibition of Elastase

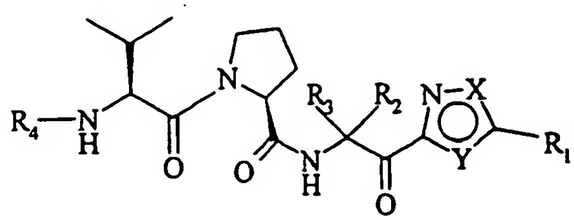
The following protocol was used to determine inhibitory activity of compounds described herein. The elastase used in the protocol was derived from human sputum (HSE). A mother solution of the HSE enzyme was prepared from commercially available HSE (875 U/mg protein, SE-563, Elastin Product Co., Inc, Missouri, USA) by diluting with saline to 1,000 U/ml, which was further diluted to 2 U/ml at 0°C prior to use.

A solution was prepared by mixing 100 µl 0.2 M HEPES-NaOH buffer (pH 8.0), 40 µl 2.5 M NaCl, 20 µl 1% polyethyleneglycol 6000, 8 µl distilled water, 10 µl of a DMSO solution of inhibitor and 2 µl solution of N-methoxysuccinyl-Ala-Ala-Pro-Val-p-nitroaniline (at concentrations of 100, 200 and 400 µM). The solution was incubated for 10 minutes at 37°C. To this was added an enzyme solution of HSE (elastase derived from human sputum). The resulting mixture was subjected to the following rate assay.

Optical density (SPECTRA MAX 250, Molecular Devices) at 405 nm due to p-nitroaniline generated by the enzyme reaction was measured at 37°C in order to measure the reaction rate during the period that the production rate of p-nitroaniline remains linear. The rate, mO.D./min., was measured for 10 minutes at 30 second intervals immediately after the addition of the enzyme solution. IC_{50} values were determined by log-logit method and converted to K_I values by Dixson plot method. The compounds are presented in Table 2 showing the inhibition activity (K_I values, nM) against HNE.

We claim:

1. A compound of the formula



wherein

X and Y are independently O or N;

R_1 is a,a-dialkylalkylaryl or a,a-dialkylalkyl fused aryl-cycloalkyl wherein the cycloalkyl group is optionally substituted with two or more O atoms;

R_2 and R_3 are independently H or alkyl; or together form a cycloalkyl ring consisting of 3-5 carbons in which one or more carbon atoms of the ring is optionally replaced with a heteroatom selected from O, S or N wherein N is optionally substituted with H or alkyl; and

R_4 is alkyloxycarbonyl;

with the proviso that, if X is O and Y is N, then R_1 is not α,α-dialkylalkylaryl.

2. The compound of claim 1 wherein X is N and Y is O.

3. The compound of claim 2 wherein R_4 is methyloxycarbonyl.

4. The compound of claim 3 wherein R_2 is isopropyl and R_3 is H.

5. The compound of claim 4 wherein R_1 is alkyl.

6. The compound of claim 4 wherein R_1 is a,a-dialkylalkylaryl.

7. The compound of claim 4 wherein R_1 is α,α-dialkylalkyl fused aryl-cycloalkyl wherein the cycloalkyl group is substituted with two O atoms.

8. A method of inhibiting at least one serine protease comprising administering to a host in need of such inhibition an effective amount of a compound of claim 1.

15. The compound of claim 14 wherein R_2 is isopropyl and R_3 is H.

16. The compound of claim 15 wherein R_1, is alkyl.

17. The compound of claim 16 wherein R_1 is *tert*-butyl and R_5-A- is selected from H, -NH_2, and benzyl oxycarbonylamino.

18. A compound of claim 17 selected from:

2-[6-oxo-2-(4-fluorophenyl)-1,6-dihydro-l-pyrimidinyl]-N-[1-(2-[5-*tert*-butyl-1,3,4-oxadiazolyl]carbonyl)-2-(*R,S*)-methylpropyl]acetamide;

2-[6-oxo-2-(4-fluorophenyl)-1,6-dihydro-l-pyrimidinyl]-N-[1-(2-[5-*tert*-butyl-1,3,4-oxadiazolyl]carbonyl)-2-(*R*)-methylpropyl]acetamide; or

2-[6-oxo-2-(4-fluorophenyl)-1,6-dihydro-l-pyrimidinyl]-N-[1-(2-[5-*tert*-butyl-1,3,4-oxadiazolyl]carbonyl)-2-(*S*)-methylpropyl]acetamide.

19. The compound of claim 15 wherein R_1 is α,α-dialkylalkyl fused aryl-cycloalkyl wherein the cycloalkyl group is substituted with two O atoms.

20. The compound of claim 19 wherein R_1 is α,α-dimethyl-3,4-methylenedioxybenzyl and R_5-A- is selected from H, -NH_2, and benzyl oxycarbonylamino.

21. A compound of claim 20 selected from:

2-[5-(Benzyloxycarbonyl)amino-6-oxo-2-(4-fluorophenyl)-1,6-dihydro-1-pyrimidinyl]-N-[l-(2-[5-(α,α-dimethyl-3,4-methylenedioxybenzyl)-1,3,4-oxadiazolyl]carbonyl)-2-(*R,S*)-methylpropyl]acetamide;

2-[5-(Benzyloxycarbonyl)amino-6-oxo-2-(4-fluorophenyl)-1,6-dihydro-1-pyrimidinyl]-N-[l-(2-[5-(α,α-dimethyl-3,4-methylenedioxybenzyl)-1,3,4-oxadiazolyl]carbonyl)-2-(*R*)-methylpropyl]acetamide; or

2-[5-(Benzyloxycarbonyl)amino-6-oxo-2-(4-fluorophenyl)-1,6-dihydro-1-pyrimidinyl]-N-[l-(2-[5-(α,α-dimethyl-3,4-methylenedioxybenzyl)-1,3,4-oxadiazolyl]carbonyl)-2-(*S*)-methylpropyl]acetamide.

22. A compound of claim 20 selected from:

25. A compound of claim 20 selected from:

2-[6-oxo-2-(4-fluorophenyl)-1,6-dihydro-1-pyrimidinyl]-N-[l -(2-[5-(α,α-dimethyl-3,4-methylenedioxybenzyl)-1,3,4-oxadiazolyl]carbonyl)-2-(*R,S*)-methylpropyl]acetamide;

2-[6-oxo-2-(4-fluorophenyl)-1,6-dihydro-1-pyrimidinyl]-N-[l -(2-[5-(α,α-dimethyl-3,4-methylenedioxybenzyl)-1,3,4-oxadiazolyl]carbonyl)-2-(*R*)-methylpropyl]acetamide;

2-[6-oxo-2-(4-fluorophenyl)-1,6-dihydro-1-pyrimidinyl]-N-[l -(2-[5-(α,α-dimethyl-3,4-methylenedioxybenzyl)-1,3,4-oxadiazolyl]carbonyl)-2-(*S*)-methylpropyl]acetamide.

26. A compound of claim 20 selected from:

2-[6-oxo-2-phenyl-1,6-dihydro-1-pyrimidinyl]-N-[1-(2-[5-(α,α-dimethyl-3,4-methylenedioxybenzyl)-1,3,4-oxadiazolyl]carbonyl)-2-(*R,S*)-methylpropyl]acetamide;

2-[6-oxo-2-phenyl-1,6-dihydro-1-pyrimidinyl]-N-[1-(2-[5-(α,α-dimethyl-3,4-methylenedioxybenzyl)-1,3,4-oxadiazolyl]carbonyl)-2-(*R*)-methylpropyl]acetamide; or

2-[6-oxo-2-phenyl-1,6-dihydro-1-pyrimidinyl]-N-[1-(2-[5-(α,α-dimethyl-3,4-methylenedioxybenzyl)-1,3,4-oxadiazolyl]carbonyl)-2-(*S*)-methylpropyl]acetamide.

27. The compound of claim 15 wherein R_1 is α,α-dialkylalkylaryl.

28. The compound of claim 27 wherein R_1 is α,α-dimethylbenyzl.

29. A compound of claim 28 selected from:

2-[6-oxo-2-phenyl-1,6-dihydro-l-pyrimidinyl]-N-[1-(2-[5-(α,α-dimethylbenzyl)-1,3,4-oxadiazolyl]carbonyl)-2-(*R,S*)-methylpropyl]acetamide;

2-[6-oxo-2-phenyl-1,6-dihydro-l-pyrimidinyl]-N-[1-(2-[5-(α,α-dimethylbenzyl)-1,3,4-oxadiazolyl]carbonyl)-2-(*R*)-methylpropyl]acetamide; or

2-[6-oxo-2-phenyl-1,6-dihydro-l-pyrimidinyl]-N-[1-(2-[5-(α,α-dimethylbenzyl)-1,3,4-oxadiazolyl]carbonyl)-2-(*S*)-methylpropyl]acetamide.

30. A compound of claim 28 selected from:

2-[6-oxo-2-(4-fluorophenyl)-1,6-dihydro-1-pyrimidinyl]-N-[1-(2-[5-(α,α-dimethylbenzyl)-1,3,4-oxadiazolyl]carbonyl-2-(*R,S*)-methylpropyl]acetamide;

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/12354

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A61K 38/05, 38/06; C07K 5/062, 5/065, 5/078, 5/097
US CL :514/18, 19; 530/331; 544/30
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/18, 19; 530/331; 544/300

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, CHEMICAL ABSTRACTS
search terms: serine protease, elastase, inhibit, oxadiazole, thiadiazole, triazole, structures of claims 1 and 12

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 5,618,792 A (GYORKOS ET AL) 08 April 1997 (08/04/97), see columns 39-40, claims 1-7 and 9-12.	1-34
A	US 5,807,829 A (GYORKOS ET AL) 15 September 1998 (15/09/98), see claims 1-17.	1-11
A	US 5,861,380 A (GYORKOS ET AL) 19 January 1999 (19/01/99), see claims 13, 14, 43-68.	12-34

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document published on or after the international filing date

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

17 AUGUST 1999

Date of mailing of the international search report

09 SEP 1999

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks

Authorized officer
L. Lawrence Jr.